Expert Opinion





# Current aspects of formulation efforts and pore lifetime related to microneedle treatment of skin

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***Importance of the field:*** The efficacy of microneedles in the area of transdermal drug delivery is well documented. Multiple studies have shown that enhancement of skin permeation by means of the creation of microscopic pores in the stratum corneum can greatly improve the delivery rates of drugs. However, skin pretreatment with microneedles is not the only factor affecting drug transport rates. Other factors, including drug formulation and rate of micropore closure, are also important for optimizing delivery by this route.
***Areas covered in this review:*** This review aims to highlight work that has been done in these areas, with an emphasis on drug formulation parameters that affect transdermal flux.
***What the reader will gain:*** This review creates an appreciation for the many factors affecting microneedle-enhanced delivery. Most results clearly indicate that microneedle skin pretreatment by itself may have different effects on drug transport depending on the formulation used, and formulation characteristics have different effects on the transport through untreated skin and microneedle-treated skin. Several formulation approaches are reported to optimize microneedle-enhanced drug delivery, including co-solvent use, vesicular, nanoparticulate and gel systems.
***Take home message:*** In addition to well-established factors that affect microneedle-assisted delivery (geometry, type of microneedle, etc.), formulation and pore viability are also critical factors that must be considered.

**Keywords:** diffusion, formulation, microneedle, micropores, transdermal

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## 1. Background/introduction

Oral drug delivery is not optimal in many situations for reasons that include gastrointestinal side effects, extensive first-pass metabolism, enzymatic degradation and poor bioavailability. A common alternative is to deliver the drug by means of injection with a hypodermic needle, which is painful, invasive and less convenient for the patient. Transdermal drug delivery, by way of patches that adhere to the skin and deliver a consistent amount of drug over an extended period of time, would be a more appealing option for most patients. There are nearly 20 different transdermal delivery systems available in the US, delivering a wide variety of drugs including fentanyl, nicotine, lidocaine, and various hormones (e.g., estradiol, testosterone) [1]. Despite its advantages, the clinical utility of transdermal drug delivery is greatly limited by the fact that most drugs are not able to cross the stratum corneum (SC), the outermost layer and primary permeation barrier of the skin [2]. With passive transdermal systems that are available at present, only drug molecules up to a few hundred Daltons can be delivered to achieve therapeutically relevant concentrations, and administration of highly hydrophilic compounds is difficult at best [1,3]. Multiple approaches have been used in an attempt to enhance the delivery

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**Article highlights.**

- Microneedle application creates micropores in the skin, thus providing a minimally invasive means of bypassing the stratum corneum barrier and increasing the number of drug molecules that can be transdermally delivered.
- Several parameters can ultimately affect the degree of drug delivery and skin permeability associated with microneedle-assisted transdermal delivery.
- Highly soluble, ionized species have been shown to increase substantially drug delivery rates as compared with their low-solubility, neutral counterparts.
- At high drug concentrations, nonlinearity in *in vitro* transport rates and *in vivo* exposure has been observed.
- Optimization of drug-containing nanoparticulate systems presents many challenges and more thorough studies are needed to elucidate their behavior in the dermis.
- Studies involving microneedle coating and polymer microneedles demonstrate the potential of formulation in tailoring drug release rates for immediate or controlled absorption.
- Owing to the low diffusional resistance of microneedle-treated skin, a shift in the transport rate-limiting step from skin to transdermal device may occur.
- Short lifetime of the pores created following microneedle application limits the clinical utility of this approach.
- Continued optimization of several components related to microneedle-assisted transdermal delivery is necessary to develop drug delivery systems that can be applied for a clinically relevant period of time.

This box summarizes key points contained in the article.



of drugs by means of the transdermal route, including (but not limited to) iontophoresis, ultrasound, chemical permeation enhancers and microneedle arrays [1,3-9].

Microneedles (MN) are a minimally invasive means of assisting the transport of drug molecules across the skin, and several reports have demonstrated that MN treatment is painless and well tolerated by most patients [1,10-13]. There are various ways that microneedles can assist in the transdermal delivery of drugs across the SC, and four major types of microneedle system have been described [3]. Solid microneedles pierce the skin and create pores (also referred to as microchannels) in the SC, which can be utilized to increase delivery of a variety of compounds by applying a gel formulation or transdermal patch over the MN-treated skin; this is called the 'poke and patch' method [1,4]. Microneedles can be coated with various drug formulations, which have been used to deliver DNA, proteins and virus particles; this is referred to as the 'coat and poke' approach [1,4]. Polymeric microneedles have been prepared with encapsulated drug to be inserted, detached and left in the skin, which can be used for either rapid or controlled release into the skin [3]. Finally, hollow microneedles have been utilized to deliver drug compounds (insulin, vaccines) by diffusion or pressure-driven flow through the bore of the needle [1,3,4]. All of these applications are inherently different and it can be expected that each technique requires separate optimization.

There are many factors that affect drug delivery and permeability of the skin with respect to MN application. These include parameters such as mechanical properties of the MN arrays, formulation of drugs to be delivered, and lifetime of the pores following MN application. So far, the mechanical properties of microneedle arrays have been described well in the literature (many of these papers were published only in the last few years), including parameters such as length, bore, sharpness, MN material, geometry, force of application and number of microneedles [10,14-18]. Complex optimization algorithms have been developed to improve skin permeability and determine the best MN geometry for both solid and hollow MN systems by examining properties such as number of microneedles, MN radius, MN length, MN patterns (square, triangular, diamond, etc.), aspect ratio and skin thickness [17,19]. Also, parametric analyses have been performed to examine the delivery of high-molecular-mass molecules from a MN system by performing quantitative analyses of various parameters, including blood concentrations of drug [18]. The optimization of these mechanical factors affects the strength of the microneedles, the amount of pain associated with application, and the overall permeability of the skin (and therefore efficiency of drug delivery) [3,17,18]. An extensive discussion of the mechanical properties of microneedles is beyond the scope of this article; however, these properties deserve note, as they do significantly affect the amount of drug delivered with a MN-assisted system and have received a great deal of attention in the recent literature. In great contrast to the mechanical properties of the MN arrays, the other factors affecting drug delivery by means of this route remain largely unexplored, specifically drug formulation and pore lifetime following MN treatment. This review describes the current literature available addressing these topics and how that information may be applied to improving drug delivery associated with MN arrays, with special emphasis on solid microneedles.

## 2. Effects of ionization, co-solvents and concentration on drug delivery

The charge of a drug can affect drug physicochemical properties in a variety of ways, which may ultimately affect drug permeation through the skin. Thus, pH of the drug formulation may have a substantial effect on rates of transdermal delivery. Banks *et al.* assessed the effects of drug ionization on delivery rates of naltrexone and its active metabolite, naltrexol, through MN-treated and untreated guinea-pig skin by comparing different pH values of aqueous formulations [20]. Both naltrexone and naltrexol possess two ionizable groups – an aliphatic nitrogen and a phenolic group. The p$K_a$ values for naltrexone are 8.2 and 9.6 and for naltrexol are 7.4 and 9.4 [20,21]. The results demonstrated that the ionized form of naltrexol (at pH 4.5) was >2 orders of magnitude more

soluble compared with the predominantly unionized form in the donor solution (at pH 8.5). Similarly, naltrexone also showed significant solubility improvement owing to shifting the formulation pH value from 8.5 to 4.5.

The comparison of transdermal flux of charged and predominantly uncharged drugs through either MN-treated versus untreated skin is also important. Naltrexol in its poorly soluble unionized form displayed only modest flux enhancement after MN treatment. However, in the highly soluble charged state, naltrexol not only permeated across untreated skin much faster, but also showed a greater flux improvement post MN treatment. This suggests that, in the diffusion through untreated skin, the high concentration of drug in the donor solution (with corresponding non-rate-limiting dissolution rate) overrides lower apparent permeability coefficient of the charged drug. Also, the greater average MN flux enhancement for the ionized form of naltrexol as compared with the unionized form indicates that the selectivity of charged molecules to diffuse through microchannels is greater than through the intact skin around the microchannels. This is not surprising as it is expected that more lipophilic, uncharged molecules would still be able to permeate through intact skin around the microchannels, but it may prove much more difficult for their charged counterparts to partition into and diffuse across the SC. On the other hand, the charge of naltrexone did not seem to have much influence on the transport through MN-treated skin (flux values were not statistically different). Rather, it is likely that the drug solubility in the donor solution plays a critical role in augmenting transport through MN-treated skin by providing a larger concentration gradient across the skin. It is noteworthy that this study demonstrated the same direction but not the same magnitude of the effect of pH formulation on the transport through intact skin and MN-treated skin, despite the different routes of penetration involved.

Also looking at the effects of formulation on the delivery of naltrexone hydrochloride (NTX•HCl) through untreated and MN-treated Yucatan miniature pig skin, Milewski and Stinchcomb investigated the influence of propylene glycol (PG)–water binary mixture donor solutions on the rates of drug transport through microchannels (data submitted for publication). Several donors containing a constant concentration of NTX•HCl in binary mixtures of PG and water were prepared. In MN-treated skin, transdermal transport proved to be a function of the donor solution composition, with the lowest flux obtained from a pure PG solution and the highest flux obtained from a pure aqueous solution, with the extent of flux difference obtained from MN-treated skin reaching as much as ~ 40-fold. With the increasing PG content in the donor solution, flux gradually dropped in a nonlinear fashion. For intact, non-MN treated skin, the flux values were much lower for all donor solution compositions. Despite a qualitative trend seen in untreated skin showing that water-rich donor solutions provide higher flux over PG-rich donor solutions, this trend does not mimic that seen with MN-treated skin. The effect of donor solution composition is different and had a much more pronounced effect on MN-treated skin as compared with the untreated skin. The data were also analyzed using a model to take into account two parallel and independent permeation pathways: one through intact skin around the microchannels and another through the microchannels. The relative importance of each pathway was shown to be dependent on the formulation used, and the nonlinear change in flux as a function of donor formulation can be rationalized on the basis of varying donor solutions' viscosities. Microchannel flux values were found to be inversely related to the donor solution viscosity. This work emphasizes the ability of co-solvents/excipients to influence percutaneous transport rates through MN-treated skin in a fashion very different from that of untreated skin, which may be helpful in the optimization of future microneedle-patch systems.

Guohua Li *et al.* investigated the transport of a model protein, IgG, using dissolvable maltose microneedles through hairless rat skin [22]. A large, 150 kDa macromolecule cannot be delivered by means of the percutaneous route without the use of an enhancement method. This study evaluated the importance of three factors on transport of IgG through MN-enhanced skin: MN length, MN number and IgG concentration in the donor solution (here only the effects of IgG concentration on transport are discussed). Donor solutions comprised of human IgG solutions at four different concentrations (in phosphate buffer pH 7.4) were evaluated. As shown in **Figure 1**, a rise in the IgG donor solution concentration caused an increase in the MN-treated skin flux in a concentration-dependent manner. Although the initial increase in the concentration from 1 to 5 mg/ml produced a proportional rise in flux (about fivefold), further change in concentration to 20 mg/ml caused flux to increase only ~ 12-fold as compared with the 1 mg/ml donor. Even more pronounced nonlinearity was evident when 40 mg/ml donor solution was compared with the 1 mg/ml donor – only ~ 11-fold difference in transdermal flux was observed. Clearly the gain in flux obtained by increasing diffusant donor concentration becomes less and less pronounced with augmenting concentration. This may imply that using very concentrated (or saturated) solutions will not provide substantial improvement (in terms of flux) over their lower concentration counterparts.

The above study used steady-state flux to compare the *in vitro* performance of different donor concentrations of the diffusant. On the other hand, Galit Levin *et al.* investigated transdermal delivery of human growth hormone (hGH, 22 kDa) through microchannels *in vivo* by using radiofrequency ablation to form microchannels in rat and guinea-pig skin [23]. Although this method is not directly related to MN use, the resulting microchannels possess characteristics similar to those obtained after MN treatment – hydrophilic nature, extending over epidermal thickness, circular geometry and very small fractional area in the experimental set-up (<1%). The focus of this publication

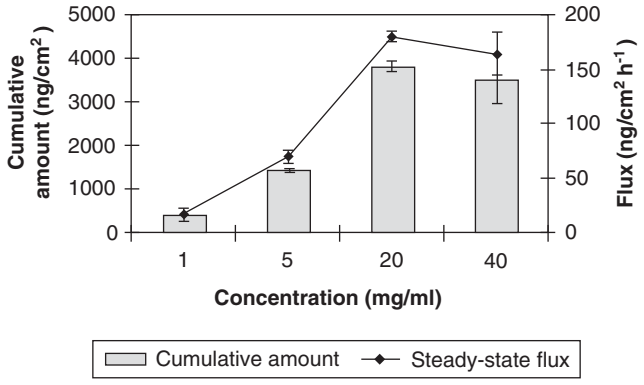


**Figure 1. Cumulative amounts of human IgG permeated and steady-state flux across hairless rat skin pretreated with two-layered microneedles (54 needles, 500 μm long) during 24 h of transdermal delivery with 200 μl of donor at 40, 20, 5 or 1 mg/ml of human IgG concentrations (mean ± s.e.) (n = 3).**

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was to examine the bioavailability (relative to subcutaneous injection) and bioactivity of hGH by using different dose patches *in vivo*. The patches containing hGH in a thin, dry layer were placed on the microchannel-affected area of animal skin. The resulting plasma profiles of hGH resembled those obtained after subcutaneous injection rather than steady-state conditions, suggesting that the SC was no longer the rate-limiting step in transport. This *in vivo* study demonstrated that, in both rat and guinea-pig, the total exposure to the hGH (as measured by AUC) was linearly dose-dependent in the lower concentration range (50 – 300 μg/patch), but further increasing the dose to 400 – 450 μg/patch did not translate into proportional exposure. This is also expressed in terms of bioavailability, which remains at a steady level over the 50 – 300 μg/patch range but drops at higher doses. Therefore, this trend mimics *in vitro* findings of the previous publication, although the underlying reasons may be different. The authors evoked factors such as dissolution rate of hGH from the patches, diffusion rate through the channels, microchannel closure process and metabolism to explain tentatively the decrease in bioavailability at high doses. Overall, however, the bioavailability of hGH was found to be very high relative to subcutaneous injection: ~ 75% in rat and ~ 33% in guinea-pigs. High bioavailability and retention of the hGH bioactivity during the patch manufacturing process indicate that this delivery route may become of practical clinical importance.

## 3. Liposomes and nanoparticles

The pores created by MN pretreatment allow enhanced percutaneous transport of molecules that permeate slowly through intact skin, and also allow the transport of marcomolecules and nanoparticlulate systems normally deemed too large to permeate at all. The micrometer-scale diameter of the pore opening allows nanometer-scale particles to diffuse into the microchannel. The publications reviewed below demonstrate some formulation efforts to evaluate the influence of certain factors such as the size, composition and charge of particles on the transport rates across skin. The nature of the diffusing particle may have a significant effect on the results, and therefore data analysis should be cautious. For example, if the main focus is placed on the drug encapsulated in the particle alone rather than both the drug and the particle it may be very difficult to gain mechanistic understanding of the transport phenomena. Liposomal formulations are complex systems and one of the unanswered questions is whether liposomes can diffuse into and through the viable epidermis/dermis in intact form, or whether they break down and release encapsulated drug [24]. Only detailed analysis of both drug and liposome constituents in skin could clarify the mechanism of drug-bearing liposomes' transport through MN-treated skin. On the other hand, the data interpretation coming from the use of solid nanoparticles where the nanoparticles themselves are quantified is more straightforward.

To evaluate the performance of different liposomal formulations across untreated and MN-treated skin, Yuquin Qiu *et al.* studied the permeation of docetaxel in various elastic liposomal formulations, with the goal of increasing skin permeation of a poorly water soluble (6 – 7 μg/ml), lipophilic (logP = 4.1) and fairly large molecule (molecular mass = 807.9 Da) [25]. The authors used four different liposomal formulations prepared by a thin film method: two formulations (F1 and F2) were elastic liposomes, whereas the remaining two (F3 and F4) were conventional liposomes. The entrapment efficiency for all four formulations was ~ 80 – 88% and the particle sizes were 43 ± 2 (F1), 197 ± 1.5 (F2), 173 ± 3.1 (F3) and 5697 ± 168 nm (F4) with polydispersity index 0.08 ± 0.01, 0.35 ± 0.01, 0.41 ± 0.01 and 1.00 ± 0.00, respectively. Prepared donor solutions were compared in an *in vitro* diffusion experiment with untreated or 150 μm MN-treated porcine skin. An experiment using untreated skin revealed that the elastic liposomes provided greater transdermal flux of docetaxel when compared with the conventional liposomal formulations, which performed poorly. On the other hand, an experiment with MN-treated porcine skin revealed different behavior of all formulations tested, as presented in **Figure 2**. First, MN treatment by itself allowed the control solution of docetaxel to permeate the skin, demonstrating the effect of MN treatment alone. Second, all liposomal formulations increased further the percutaneous flux, irrespective of the liposomal formulation, and produced similar steady-state flux. Also, the size of the liposomes varied greatly from formulation to formulation and yet this did not affect the outcome. One significant difference that was noted was the lag time. Elastic liposome formulations (F1 and F2) had lag times ~ 4 h, whereas conventional liposomes (F3 and F4) had lag times ~ 14 h. The authors set

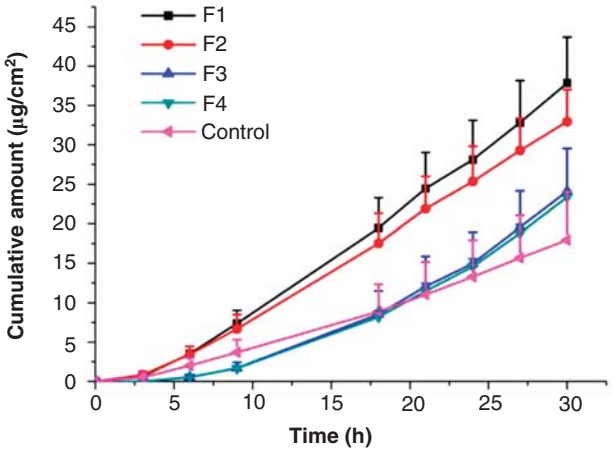


**Figure 2. Penetration of docetaxel from liposomal formulations and control saturated 20% w/w ethanolic solution through porcine skin treated with microneedles (n = 4).**
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forth a hypothesis to explain the difference in terms of the ability of elastic and inability of conventional liposomes to release rapidly the drug in the microchannels into viable tissue. This cannot be concluded with certainty as the fate of conventional and elastic liposomes was not explicitly followed in the skin. Nevertheless, from a practical point of view, this investigation clearly demonstrated how the performance of four formulations and a control solution was changed by MN treatment of the skin. Not only did the flux increase substantially, but also the changes were different for each class of donors tested: elastic liposomes, conventional liposomes and drug solution.

Badran *et al*. investigated the effect of microneedle size and applied formulation on the *in vitro* permeation of the model hydrophilic drug mannitol [26]. In this study, microneedle lengths of 150, 500 and 1500 µm were evaluated; also, the effect of a colloidal drug carrier system – invasomes – was tested. Invasomes are highly flexible vesicles that contain permeation enhancers such as ethanol and terpenes besides phospholipids. This study evaluated the performance of invasomes in combination with MN skin perforation, hypothesizing that MN treatment may further facilitate transport of the invasomes across skin. Mannitol-loaded invasomes obtained by extrusion procedure were 123.6 ± 0.3 nm in diameter, with a polydispersity index of 0.08 ± 0.01. The results from 6-h-long *in vitro* diffusion experiments presented in terms of the per cent of the applied dose that was found in the receiver solution are shown in **Figure 3**. First, without MN treatment of the skin, invasomes were able to deliver a small quantity of mannitol (0.4%) across the skin. Second, diffusion of invasomes through microchannels enhanced the transport further. The results also demonstrated that the magnitude of enhancement differed depending on the MN length used. Longer microneedles correlated with greater amount of diffusant found in the receiver solution after 6 h, ranging from 18% (1500 µm MN) to 11% (500 µm MN) and 5% (150 µm MN). In other words, the length of microchannels formed after MN treatment was not of critical importance when combined with a buffer formulation, but the effect was much more pronounced with the invasome formulation. It should be noted, however, that these authors used invasome formulations without separation of the drug contained in the outer water phase. This further confounds the data analysis as at least two different species – drug in invasomes and free drug – are present in the donor solution. The authors suggest that the flexible vesicles can enhance the penetration of the drug dissolved in the outer water phase in addition to invasomes fusing or aggregating with epidermal cells, hence augmenting their flow towards the epidermis from the donor solution. It seems that an alternative explanation could involve the difference in the diffusion coefficients of free drug and invasomes between dermis and solution in the microchannels. For free drugs the diffusivity in the viable tissue is usually up to 10 times lower as compared with aqueous milieu [2]. On the other hand, if the diffusivity of invasomes drops more substantially while in dermis, and assuming they can diffuse intact throughout it, a greater sensibility of the vesicular flux to the microchannel length would be expected, as shown in **Figure 3**. Despite the difficulties in the interpretation of the data, the experiments showed a pronounced effect of the formulation on the per cent of the applied dose detected in the receiver solution, with invasomes being superior in this respect.

Coulman *et al*. studied the microneedle-mediated delivery of nanoparticles into human skin and through porous membranes [27]. The purpose of the study was to evaluate the effect of artificial porous membrane surface charge and pore size on the permeation and to study the transport across MN-treated skin. Fluorescent polystyrene nanospheres served as model nanoparticles (NPs) and were characterized by

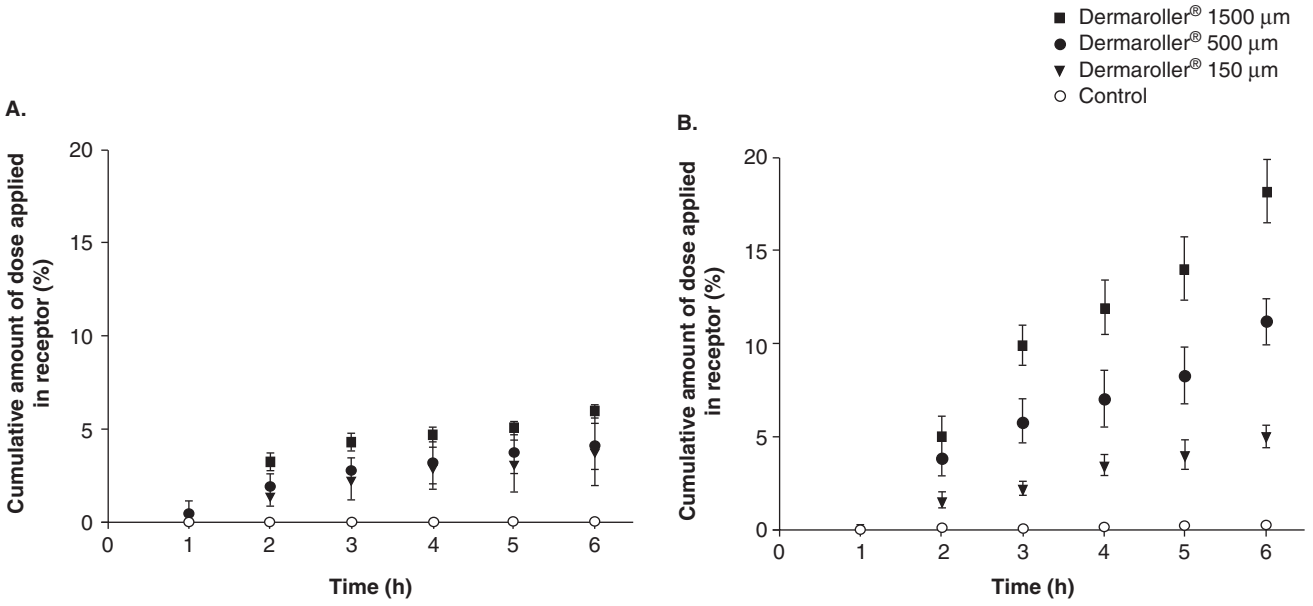


**Figure 3. Cumulative amount of radiolabeled mannitol in the receptor compartment on the formulation (A. buffer solution; B. invasomes) and Dermaroller® treatment (n = 3).** The controls present the skin samples without Dermaroller treatment.
Reproduced with permission from [26].



hydrodynamic diameter, zeta-potential and surface morphology. The mean NP diameter measured by photon correlation spectroscopy was found to be 138 ± 25.1 nm. The pH of the NP formulation is important for at least two major reasons. First is the change of the surface charge with a variation in pH. It was shown that particles that do not possess surface charge (zeta-potential) high enough to provide electrostatic repulsion are prone to aggregation and are hence instable. The second is a potential for charge-dependent NP-membrane/skin interactions that may affect the transport. Therefore, the authors decided to use pH as a means to control the surface charge of the NPs, and showed that two units below the isoelectric point at pH 5 they were positively charged (~ 40 mV), whereas two units above pH 5.0 they were negatively charged (~ -40 mV). To eliminate biological variability, the first set of experiments used the Isopore® (Millipore, Billerica, MA, USA) membranes, which are considered to be surrogates for the MN-treated skin rather than the MN-treated skin itself. Membrane characteristics include cylindrical pore shape, three different pore sizes (100 nm, 1.2 µm and 10 µm) and low negative surface potential. Also, pH values of 7.4 and 3 were chosen to investigate the influence of NP charge on the transport through pores as they result in a zeta-potential of -40 and 40 mV, respectively. At physiological pH 7.4 NPs diffused rapidly through 10 µm pores but the rate of transport through 1.2 µm pores was substantially reduced, and the NPs were not able to permeate through 100 nm pores at all. The authors explained the observed phenomenon by evoking a greater hindrance the NPs may experience diffusing through the pores of 1.2 µm diameter as compared with 10 µm. Alternatively, a change in the charge density on the internal size of individual microchannels was hypothesized to have an effect on the rate of permeation. The complete lack of detectable NPs in the receiver solution after using a membrane with 100 nm pores was believed to be due to a simple size exclusion principle. Next, the same experiments were repeated at pH 3.0. The reverse in the zeta-potential caused a marked change in the outcome. For 10 µm pores, the NP permeation was diminished as compared with pH 7.4; moreover, for pores of size 1.2 µm and 100 nm, no NPs were detected in the receiver solution. The authors suggested that these differences in transport were largely due to the surface charge-dependent NP–membrane interactions. For 10 µm pores, the pore diameter was sufficiently large to diminish their importance; however, for 1.2 µm it would be responsible for 'immediate absorption of nanoparticles to the membrane and rapid accumulation, resulting in occlusion of microchannels'. Mass balance showed that as much as 56% of the applied formulation at pH 3.0 was not detected in either donor or receiver solution, which contrasts sharply with only 10% for pH 7.4. Based on this observation, as well as the increased membrane fluorescence detected at pH 3.0, the authors put forward a hypothesis that the electrostatic interactions between positively charged NPs and the negatively charged membrane surface are responsible for altered transport of NPs at this pH. Finally, a diffusion of 100 nm NPs through MN-treated human epidermis was investigated. Both MN treatment and hypodermic needle treatment of the epidermis resulted in elevated transport of NPs as compared with the

untreated epidermis. Scanning electron micrographs revealed that, in this experimental set-up, the microchannel diameters created by either MN or hypodermic needles were of approximately the same size, 50 – 100 μm. However, the number of such microchannels was greater for MN-treated skin. In this context, it is not surprising that MN treatment afforded a greater percentage of the applied dose to permeate to the receiver solution as the fractional skin area corresponding to channels was also higher. Interestingly, this study indicates that whole NPs can diffuse through MN-treated epidermis. However, it is not clear whether epidermal sheets were fully perforated by the use of MN. Such a case would practically result in direct contact of donor solution with receiver solution with no viable tissue in-between them. Therefore, the appearance of the NP formulation in the receiver would imply the ability of NPs to diffuse through the channels rather than through the combination of channels and viable tissue underneath. Also, as the authors admit, the error associated with measurements is large, thus further complicating the analysis of the data. Nevertheless, experiments carried out with Isopore membranes point out that even in a relatively simple system devoid of biological variability, the electrostatic interactions between charged NPs and porous membrane may play a substantial role in the permeation characteristics of a particulate formulation. Further studies are needed to elucidate its importance in the experiments with MN-treated skin.

McAllister *et al.* studied the delivery of macromolecules and nanoparticles through MN-treated skin [28]. The hypothesis under scrutiny was that MN can create transport pathways that allow small and large drugs and nanoparticles to be delivered transdermally without pain. Apart from the engineering aspects of MN fabrication and characteristics, the authors also described *in vitro* diffusion experiments in which a variety of model compounds of different molecular size were used. Calcein, insulin, bovine serum albumin (BSA) and polystyrene latex nanospheres of 25 and 50 nm were chosen to provide a wide spectrum of molecular/particular radius. A model was used that described the apparent permeability coefficients in terms of fractional area of the microchannel pathway, molecular diffusivity in the microchannels, and length of the microchannels in combination with the Einstein–Stokes equation, and recognizing possible hindrance to diffusion of nanoparticles within annular gaps. This allowed correlation of molecular size (radius) with skin permeability. The intact skin was assumed to be effectively impermeable to diffusants. Also, two cases were compared – MN inserted and left in the skin, and MN inserted and then removed from the skin. The first case resulted in the creation of annular gaps in-between MN and surrounding skin, whereas the second case produced residual microchannels in the skin. Electron microscopic measurements afforded calculation of the fractional area available for diffusion in each case. The results of *in vitro* experiments and calculated permeability coefficients are summarized in **Figure 4**. Experimental data agreed well with the predicted permeability values over the molecular radius span of ~ 2 orders of magnitude. This supports the idea that transport indeed occurs across aqueous microchannels created with the help of MN in accordance with a simple model proposed. In this study the authors used fully perforated human epidermal membranes and the length of the channels was taken to be 50 μm. Hence, the skin permeability reported corresponded to the permeability of model compounds in the microchannels alone. This clearly demonstrates the ability of diffusants as large as nanoparticles to permeate along microchannels; however, their ability to diffuse in the viable tissue was not studied at this time. Moreover, this work suggests that the drug diffusivity in the microchannels can be successfully predicted using the Einstein–Stokes equation, much as in the bulk solution.

## 4. Microneedle coating and polymer microneedles

One alternative method of MN application involves coating the MN with drug solution. This mode of MN use is quite different from other MN applications in many respects. The drug is coated directly onto the MN and does not require any type of extra reservoir or accompanying patch. However, the overall amount of drug delivered is much lower and the release profile is also expected to differ significantly from the combined MN-patch approach. The complete process involves coating of a drug solution on solid MN, insertion into skin and drug dissolution within the skin.

From a practical standpoint, it is crucial to be able to apply uniform coatings consistently with reproducible and high amount of drug. Gill and Prausnitz investigated coating formulations for microneedles in order to develop a rational and systematic approach for optimizing the coating processes, in terms of amount of drug deposited, uniformity and thickness of coating for a variety of compounds of different physicochemical properties [29]. The authors used a micro-dip-coating process to cover the surface of solid MN with drug formulation and to fill into specially designed 'pockets' within the MN. The important physical properties affecting the thermodynamics and hydrodynamics of dip-coating are surface tension and viscosity of the formulation. Given complicated kinetics of wetting and de-wetting processes involved in dip-coating, theoretical model development is difficult and the authors decided to study experimentally the surface tension and viscosity effects instead. The coating solution consisting of the model drug sulforhodamine (characterized by low viscosity and high surface tension) did not produce any coating on the microneedles. Experiments indicated that reducing the surface tension of the coating formulations by the use of surfactants such as Lutrol F-68 or Tween 20 produced uniform but thin coating sheets deposited on MN. However, a thicker coating is required to increase the drug loading. An augmented viscosity can provide such improvement by increasing the hydrodynamic drag on the liquid during the

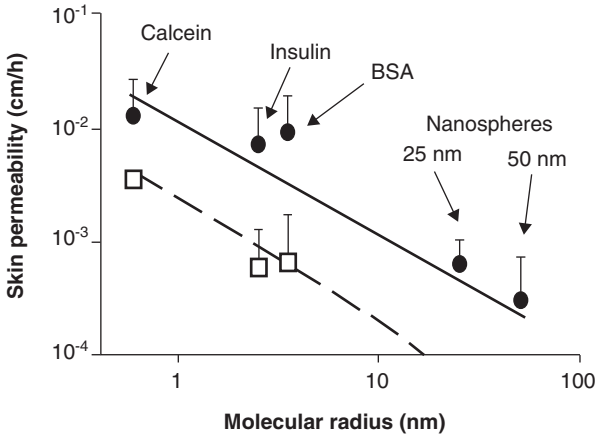


**Figure 4. Skin permeability to molecules and particles of different sizes after treatment with microneedles.** The permeability of human cadaver epidermis was increased by orders of magnitude with a 400-needle array inserted (open squares) and after the array was removed (filled circles) for calcein, insulin, BSA, and latex nanospheres of 25 and 50 nm radius.

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dipping withdrawal phase. Excipients that were successfully used for this purpose included carboxymethylcellulose (CMC) and sucrose. Overall, the combination of reduced surface tension and increased viscosity resulted in homogenously spread thick coatings. Further investigation focused on the optimization of the excipient choice for specific application, for example, to avoid disadvantageous drug–excipient interactions. More viscosity enhancers were tested in combination with Lutrol F-68, including hyaluronic acid, xanthan gum, sodium alginate, polyvinylpyrollidone and sucrose. All except sucrose produced thick uniform coatings. A formulation containing sucrose did not coat the MN tips, which was attributed to the relatively high surface tension of that solution. Other strategies facilitating the coating process included the use of organic solvents (rather than water) and modification of MN surface properties. When selectively filling 'pockets' in special MN design, both high viscosity and high surface tension proved to be advantageous and prevented the formulation from wetting the MN surface. Drugs of different physicochemical properties may require aqueous, organic or molten coating solutions for optimal coating results. Regiospecific coating and the use of multiple layers are also possible, thus affording better control over release profiles of drugs. The authors demonstrated wide potential for application of the drug-coated MN and it can be expected that the identification of the surface tension and viscosity as key parameters may greatly facilitate future work in this area.

Yet another mode of MN application involves drug-containing biodegradable MN left in the skin for prolonged, controlled release. Similarly to the previous case, the total amount of drug is limited by the microscopic dimensions of the MN. However, this approach lends itself to the possibility of better control of the release, which can be modified by the use of different encapsulation techniques.

Jung-Hwan Park *et al.* investigated polymer microneedles for controlled-release drug delivery, using poly-lactide-*co*-glycolide (PLGA) microneedles with model drugs to study the effect of single- and double-encapsulation on the release profiles [30]. Hypodermic needles are typically used in conjunction with polymeric particles to achieve controlled release of drugs in the body. The authors hypothesized that polymeric microneedles made with a drug-containing biodegradable polymer introduced into the skin could be a viable alternative. Two model compounds were included in the studies: calcein and BSA. The *in vitro* release studies were conducted using full-thickness cadaver skin. Single-encapsulation formulation consisted of directly entrapped calcein or BSA in the PLGA matrix of the MN. Both model compounds displayed release profiles consistent with a diffusion-controlled release, suggesting that no significant degradation of polymer occurred during the experiment. This was further confirmed by microscopic observation of MN after the completion of the experiment. Also, a large difference in the rate of release between two compounds of substantially different molecular masses supports this conclusion. A modified Higuchi equation, in which amount of drug released is a function of square root of time, was used to obtain apparent diffusion coefficients of calcein and BSA in the PLGA matrix of MN. These coefficients were found to be $1.2 \times 10^{-10}$ and $3.0 \times 10^{-12}$ $cm^2/s$, and contrasted sharply with much greater diffusivity of the above compounds in water, being $5.0 \times 10^{-6}$ and $5.9 \times 10^{-7}$ $cm^2/s$, respectively. Thus, the single-encapsulation greatly slowed down diffusion, which is advantageous for prolonged and controlled release. The effect of double-encapsulation was also studied. First, calcein was entrapped within CMC microparticles and then encapsulated into the PLGA microneedles. Alternatively, calcein was entrapped in the poly-L-lactide (PLA) microparticles and subsequently encapsulated within the PLGA microneedles. Each double-encapsulation caused a substantial delay of calcein release when compared with the single-encapsulation formulation. The use of CMC afforded a decrease in the release kinetics by more than one order of magnitude, with some burst effect attributed to calcein not bound to CMC present in the MN matrix. On the other hand, the use of PLA resulted in an initial burst effect followed by a very slow release (another two orders of magnitude apparent diffusivity decrease) of calcein later on. Overall, this work demonstrated the effect of single- and double-encapsulation on the release kinetics of drug from polymeric MN. It is interesting to note how well the release kinetics can be controlled by the use of techniques suggested by the authors. Also, unlike in the case of aqueous donor solutions used in most *in vitro* studies reported earlier for the 'poke and patch' method, this investigation emphasizes the rate-limiting role of the MN composition itself for the delivery of the drug into systemic circulation. These findings can help design future controlled-release MN systems.

## 5. *In vivo* pore lifetime following microneedle application

In addition to the formulation of drug to be combined with MN treatment, another important factor that can affect drug delivery is the lifetime of the pores that are created in the SC, as rapid closure of the pores would severely limit drug delivery. The lifetime of the pores created can be monitored by various methods, including transepidermal water loss (TEWL) and electrical resistance. Disruption in the barrier is accompanied by an increase in TEWL and a decrease in resistance [15,31].

Various means of disrupting the barrier function of the SC have been described extensively (in addition to MN treatment), including tape-stripping and acetone treatment. Tape stripping removes the SC mechanically, whereas acetone treatment extracts lipids from the SC [32,33]. Restoration of barrier function occurs in a similar amount of time following tape-stripping and acetone treatment, with complete recovery achieved in ~ 3 days in most cases [34,35]. In addition to the time required for barrier restoration, many laboratories have also studied the physiological mechanisms underlying the restoration of barrier function following these insults, which includes processes involving lamellar bodies, ions (calcium and potassium) and cytokines (IL-1, IL-6, tumor necrosis factor), among others [36-38].

Similar to tape-stripping and acetone treatment, MN treatment also provides a means of disrupting the barrier function of the SC. However, the time frame in which restoration of barrier function occurs and the associated physiological processes are not well understood. This topic is receiving increasing attention in the literature, and a recent publication utilized confocal laser scanning microscopy to describe the kinetics of pore closure in six healthy human subjects who were treated with microneedles. These data demonstrated that the pores do, in fact, close very rapidly (~ 15 min in most cases) [39].

One compound that is ideal for the study of MN-assisted transdermal delivery and micropore closure is naltrexone HCl (NTX), an opioid antagonist used for the treatment of opioid and alcohol dependence. NTX is an ideal candidate for the study of MN-assisted transdermal delivery because its physicochemical properties (specifically its hydrophilicity) make it difficult to deliver across intact skin, and it shows erratic bioavailability when given by means of oral administration. A recent study examined MN-assisted delivery of NTX in healthy human subjects and, as expected, NTX levels were undetectable in control subjects, confirming that NTX does not readily cross intact skin. By contrast, NTX levels in MN-treated subjects demonstrated that the pores do allow delivery of NTX across the SC. Also, results suggested that the pores remained viable for up to 48 h (under occlusion) in most subjects (72 h for 2 subjects), as plasma levels of NTX remained relatively constant during this time [40]. The authors also demonstrated that the electrical resistance of the skin dropped after microneedle treatment, followed by a gradual increase that further supported the NTX pharmacokinetic data. Another study was performed by Banks *et al.*, studying rates of pore closure in hairless guinea-pigs [41]. Hairless guinea pigs were treated with MN arrays, followed by application of an occlusive naltrexol (NTXOL, active metabolite of NTX) transdermal patch. Pore lifetime was evaluated by means of NTXOL pharmacokinetic analysis and TEWL measurements. These data demonstrated that pore closure occurred ~ 48 h following MN treatment, correlating well with the human study mentioned previously. Also, this group has data demonstrating that pore lifetime can be extended up to 7 days with daily application of Solaraze® gel (3% diclofenac sodium, a nonspecific cyclooxygenase [COX] inhibitor, PharmDerm® Melville, NY, USA), indicating a possible role of inflammatory mediators in the pore healing process [42].

Further factors that may also affect the repair of barrier function of the SC (and thus the utility of MN treatment) are hydration and occlusion. Prolonged occlusion of the skin leads to increased skin hydration, an effect that can be readily seen via TEWL and resistance measurements [43,44]. This increase in hydration can have pronounced effects on the permeability of the SC, and often increases drug penetration through the skin [44-46]. Also, water transit has been proposed to be a signal involved in stimulation of barrier repair following disruption, and may have an effect on the duration of pore lifetime [47,48]. Recent data in a hairless rat model indicated that pores close in ~ 15 h when left unoccluded, but this time frame extends to 72 h when the pores are occluded by a plastic film or solution [48]. As noted previously, treatment sites were occluded in the human study of MN-assisted permeation of NTX, and this may have positively affected the duration of pore lifetime by inhibiting the barrier recovery [40]. This effect was also noted in the study with hairless guinea pigs, as unoccluded sites appeared to restore barrier recovery much more quickly than sites that were occluded. From a clinical standpoint, current transdermal delivery systems involve complete occlusion of the skin for hours to days at a time, and thus the effects cannot be ignored. In fact, the effects of hydration and occlusion have positive implications with MN treatment, as the 'poke and patch' method of MN-assisted drug delivery involves occlusion of the newly formed pores with a transdermal drug patch. Ultimately, the combination of slowed restoration of barrier function and increased drug permeability could be useful in the clinical setting.

## 6. Conclusion

Formulation plays an important role affecting drug delivery rates through MN-treated skin. Either in the case when a maximum percutaneous flux is sought or the case when a slow, controlled release is required, the formulation is of great significance. The publications so far indicate that the

influence of a given formulation on the transport through untreated and MN-enhanced skin varies. As reviewed in this article, most authors at present focus on the *in vitro* diffusion studies. In the *in vitro* experiments the diffusive path length can be controlled by the choice of skin of a certain thickness (epidermis versus full thickness) and MN length. This path length corresponds to the distance a drug molecule has to traverse through the microchannel and viable tissue in order to reach receiver solution from the donor solution. The authors chose different experimental conditions that resulted in change not only in the rate of permeation but also in the shape of delivery profiles. Where some authors report permeation profiles indicative of reaching steady-state conditions, others report permeation profiles showing non-steady-state conditions (**Table 1**). One factor that seems to affect the shape of the profiles significantly is the thickness of the viable tissue between the microchannel and the receiver solution (authors' unpublished data). When a thick wedge of viable tissue is present the permeation profiles can be described well in terms of the steady-state flux and lag time. However, in the absence of the viable tissue under the microchannel (skin fully perforated), profiles show initial high permeation rates that taper off at a later time. This underlines the importance of experimental condition selection.

Overall, multiple strategies can be used to achieve maximum flux through MN-enhanced skin by the use of the 'poke and patch' method. First, the formulation should not be rate-controlling. Simply increasing the drug donor concentration results in an elevated concentration gradient through the skin and hence augmented delivery rates are expected. For ionizable drugs, pH control or salt formation may be the simplest tool to achieve this goal. However, at high drug concentrations substantial deviations from ideal behavior are observed and the increase in drug concentration may not be paralleled by a proportional rise in flux. Also, co-solvent use could be potentially helpful, but propylene glycol has been shown to cause marked decrease in the flux, possibly by decreasing drug diffusivity in the barrier (Milewski and Stinchcomb, data submitted for publication). Vesicular and nanoparticulate systems could be useful for compounds that fail to be formulated using a simpler method and do not permeate well through intact skin. Their performance showed a high dependence on the size of the microchannel and its length, although further studies are needed to improve understanding of the phenomena involved in their transport through skin.

Finally, the kinetics of pore closure after MN insertion and removal play a critical role in the ability of drugs to cross the SC. This has already been demonstrated by means of correlation of TEWL and pharmacokinetic data in both humans and hairless guinea-pigs [40]. In both models there appears to be a relationship demonstrating increasingly unpredictable pharmacokinetic profiles as the pores begin to close. Although MN pretreatment is certainly an effective means of increasing permeability of various drugs through the skin (especially when combined with other factors such as hydration and occlusion), maintaining the lifetime of the pores is crucial for maintaining the usefulness of this approach to drug delivery.

## 7. Expert opinion

In the diffusion experiments using untreated skin, often the influence of formulation on the transport is analyzed in terms of ideal thermodynamic behavior. A drug saturated in different solvents shows the same maximum activity in those solvents. Hence, its partitioning into the rate-limiting SC lipid domain should result in the same concentration in the membrane, and transdermal flux values obtained from such donor solutions are expected to be the same. Deviations from this behavior are approached by evoking permeation-enhancing properties of co-solvents by their ability to alter the barrier properties of the SC. In the case of MN-treated skin, the creation of microchannels allows drug molecules to bypass the SC barrier. The most relevant question seems to be what becomes of the barrier in the absence of the SC? As reported previously, the formulation itself can become the rate-limiting step in the delivery [30]. The authors showed how using drug encapsulated in polymer MN could allow the delivery rates to be controlled by the drug release from MN. In this situation the skin release profiles are described better by a modified Higuchi equation, which linearly relates mass released to the square root of time, rather than the steady-state conditions. Next, when the drug diffusion coefficients in the formulation are high (as is the case in the bulk aqueous solution) it could be expected that viable tissue will be the primary rate-limiting step in the skin permeation process. This appears to be the case for most articles reviewed here. Also, it seems that in the situation when formulation excipients can diffuse out of the vehicle and into the viable tissue they can alter its diffusional properties, as suggested by Milewski and Stinchcomb. It is likely that viscosity-increasing propylene glycol can change the microviscosity of the viable tissue and therefore decrease the mobility of drugs in this rate-limiting environment.

One aspect of the MN-assisted drug delivery that has been underrated and will be investigated more closely in the near future is the pharmacokinetics of drugs delivered with the help of a MN-and-patch method. So far, it has been typically assumed that steady-state plasma profiles will prevail after MN pretreatment of the skin. *In vivo* studies in guinea-pigs (data submitted for publication) and one study in humans have shown that the plasma drug concentrations are relatively constant [40,41]. If, under *in vivo* conditions, some formulations control the release of the drug and are rate-limiting in transport, then the plasma drug concentrations should eventually decline with time. This seems to be the case in the study reported here, where the authors used a dry thin film of drug in the patch, which resulted in plasma profiles similar to those obtained after subcutaneous injection [23].

**Table 1. Comparison of different *in vitro* diffusion experimental conditions and resulting permeation profiles.**

| Authors | Diffusion cell | Skin type | MN length | Permeation profile |
|---|---|---|---|---|
| Banks *et al*. [20] | Flow-through cell | Full-thickness hairless guinea-pig skin and full-thickness human abdominal skin | 750 μm | SS |
| Milewski and Stinchcomb | Flow-through cell | Full-thickness Yucatan miniature pig skin | 750 μm | SS |
| Li *et al*. [22] | Franz cell | Full-thickness hairless rat skin | 200 and 500 μm | SS* |
| Qiu *et al*. [25] | Franz cell | Full-thickness abdominal rat skin and 600 μm dermatomed porcine skin | 150 μm | SS |
| Badran *et al*. [26] | Franz cell | Full-thickness abdominal human skin | 150, 500 and 1500 μm | Non-SS |
| Coulman *et al*. [27] | Franz cell | Human breast epidermis | ~ 300 μm | Non-SS |

*Some other profiles not shown in this article are non-SS.
Non-SS: Non-steady-state; SS: Steady-state.

None of the above-mentioned factors takes into account the kinetics of pore closure *in vivo* and changes in the diffusional properties of the microchannel environment. So far, very limited data are available to provide clues about the lifetime of pores and mechanisms of restoring barrier function following MN treatment. Relatively rapid closure of the pores severely limits the clinical utility of MN application, and understanding the factors involved in healing the pores created by MN treatment is critical for optimizing the use of microneedles in clinical situations. Once weekly dosing of a transdermal patch would be ideal for clinical applications, requiring patients to change a patch only once every 7 days. Effects of hydration and occlusion on pore lifetime appear to be helpful in extending pore lifetime, and are a natural (and convenient) effect of the 'poke and patch' method. To continue to push the MN field forward towards clinical practice, substantial efforts need to be made to develop safe and effective means of extending pore lifetime to this end point.

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## Declaration of interest

AL Stinchcomb is a majority shareholder in AllTranz, Inc., a specialty pharmaceutical company developing COX inhibitor technology for use with MN systems.

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